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Manufacturing functional biscuits fortified with dill (*Anethum graveolens L.*) and assessing its physical and sensory properties and microbial activity

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ABSTRACT:

Background: Natural substances derived from dill (Anethum graveolens) are characterized by broad antimicrobial activity against numerous human pathogens, attributable to their rich content of bioactive compounds and strong antioxidant and antimicrobial properties. Consequently, dill may be considered both a valuable nutritional supplement and a promising source of herbal medicinal agents.

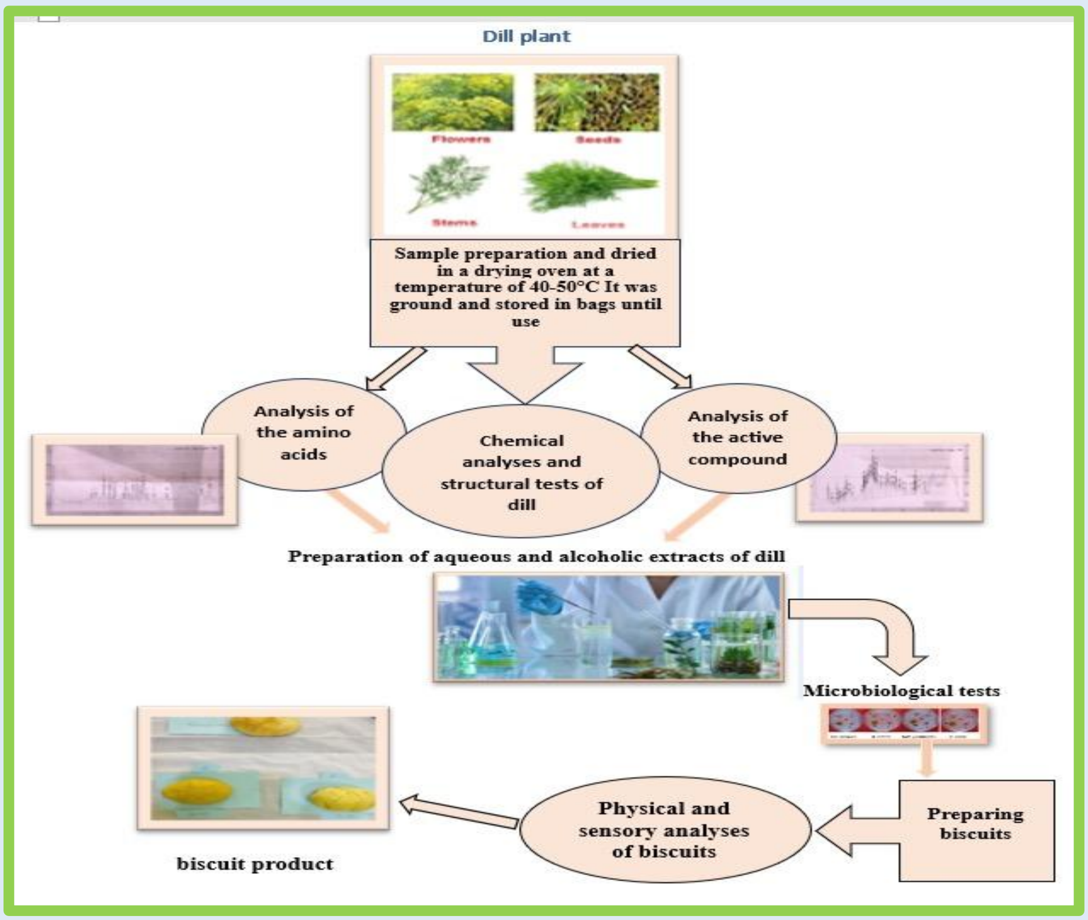
Objective: This research aimed to develop high-fiber biscuits through partial replacement of wheat flour with dill powder levels at 5% and 10%, and to evaluate the effects on chemical composition, antioxidant capacity, and antibacterial activity against selected pathogenic microorganisms.

Results: Chemical analysis of dill revealed carbohydrate, protein, fat, crude fiber, and ash contents of 9.02%, 3.26%, 2.10%, 3.12%, and 0.96%, respectively. The moisture content was 81.34%, indicting a high water-retention capacity that may positively influence both sensory and functional properties. Phytochemical analysis demonstrated a diverse profile of phenolic and aromatic compounds, as well as amino acids. Microbiological analysis confirmed that all biscuit samples were free of coliform bacteria. Additionally, the alcohol extract of dill exhibited strong inhibitory activity against the tested bacteria, including E. coli, Salmonella, and Staphylococcus. Sensory evaluation indicated that dill incorporation improved taste, aroma, texture, and overall acceptability of the biscuits compared with the control formulation.

Novelty: This study is the first to systematically incorporate dill powder into high-fiber biscuits while simultaneously evaluating its nutritional enhancement, antioxidant capacity, and antimicrobial activity against foodborne pathogens. Unlike previous studies that have focused on dill extracts or on bakery product fortification in isolation, the present work demonstrates the dual functionality of dill as both a natural preservative and a functional food ingredient within a ready-to-eat bakery product.

Conclusion: Dill exhibits valuable nutritional properties, including appreciable protein and lipid contents, in addition to serving as a rich natural source of phytonutrients. These findings highlight the multifaceted potential of dill as a source of bioactive compounds, supporting its application in the functional food industry as a natural antioxidant and preservative. Consequently, dill may serve as a safe and effective ingredient for enhancing both the nutritional value and sensory quality of food products.

Keywords: Dill powder; Functional food; Natural preservative; Plant-based fortification; High-fiber biscuits; Antimicrobial activity; Antioxidant capacity



Graphical Abstract: Production of healthy biscuits fortified with 5% and 10% dill and a study of their properties physical and sensory.

INTRODUCTION

Dill (*Anethum graveolens*) is one of the most widely used culinary and medicinal plants and belongs to the family Apiaceae, which includes more than 150 species. It is an important leafy vegetable and medicinal crop used as a condiment in a variety of foods. Dill is native to Asia Minor, Iran, and North Africa, encompassing both the eastern and western Mediterranean regions [1]. Due to its beneficial nutritional value, dill is grown worldwide, including in Iraq, and its uses have diversified, as reported by the World Health Organization. This Plant has been a prolific source of antimicrobials and natural antioxidants. Dill is characterized by a strong aroma, attributed to its volatile oil content, which may constitute up to 60% of its bioactive components. It contains a wide range of potent antioxidants that contribute to its functional and therapeutic properties [2]. The present study aligns primarily with Steps 2 and 3 of the Functional Food Center's 17-step model, focusing on the identification of bioactive compounds and the development of a functional food prototype with demonstrated antimicrobial and sensory attributes [3].

The chemical composition of dill is dominated by bioactive compounds with antimicrobial activity, particularly anethole and dillapiole. Both compounds possess aromatic and polar groups capable of forming hydrogen bonds, rendering them therapeutic efficacy in medicine [4]. These substances may also function as natural antioxidants in food systems, thereby supporting quality retention. It is well established that plant extracts exert strong inhibitory effects on microorganisms due to their high content of phenolic compounds. This characteristic makes dill well suited for food applications as a natural preservative, capable of prolonging shelf life while enhancing the nutritional and health-promoting properties of food products [5-6].

Biscuits are among the most widely consumed food products across all age groups, valued for their variety of flavors, ease of preparation, convenient packaging and transportation, and relatively low production costs compared with other food products. Increasing consumer demand for healthy, natural, and functional foods has prompted extensive research into enhancing the nutritional value of base ingredients and enrichment with natural components rich in nutrients and dietary fiber. Such approaches aim to improve functional and sensory properties without adversely affecting product quality or consumer acceptability [7-8].

The present study aimed to analyze the active compounds in the dill plant (*Anethum graveolens L.*), evaluate its inhibitory properties against some types of bacteria and study the effect of dill powder at 5% and 10% replacement rates in biscuit manufacturing on physical and sensory properties, thus contributing to the production of biscuits with higher nutritional quality and functional value. By utilizing the biologically active properties of natural dill compounds, new horizons may be opened for the development of healthy and high-quality baked goods.

MATERIALS AND METHODS

Sample preparation: Fresh dill plants were obtained from farms in Salah al-Din Governorate. The plants were washed thoroughly with running water, followed by rinsing with distilled water. The plant samples were separated, spread out on filter paper, and dried in a drying oven at 40-50°C. Then, they were ground and stored at 4°C in bags until use.

Chemical analyses*:* Approximate analysis of plants was conducted according to the method of [9]. Carbohydrate content was calculated by difference.

Analysis of the active compounds and amino acids: The active compounds were estimated according to [10]. The amino acid ratios were estimated in the dill plant using the Amino Acid Analyzer device [11].

Preparation of the aqueous extract*:* An aqueous extract of dill was prepared by mixing 25 g of dried plant material with 500 mL of distilled water using a magnetic stirrer. The mixture was allowed to stand for 24 hours filtered first through a clean cloth and subsequently through Whatman No. 1 filter paper. The filtrate was then concentrated in shallow dishes at 50℃ until the desired consistency was achieved.

Preparation of the alcoholic extract: Twenty-five grams of dried sample were extracted using 80% ethyl alcohol. After soaking, the mixture was allowed to stand for one hour, then filtered and concentrated using a rotary evaporator at 40°C. The extract was poured into dishes and left to dry in oven at 50°C. The powder was then skimmed off and placed in dry glass tubes covered with aluminum foil until use [12]. After sterilization, stock solution was prepared by dissolving 1 gram of powder in 5 ml of distilled water to make a concentration of 200 mg/mL. The solution was sterilized by filtration using 0.4-micron membrane filters, used after diluted to a concentration of 50 mg/mL.

Antibacterial tests: Experimental microbiological tests were conducted, in which 3 types of Gram positive and two types of Gram-negative bacteria were selected as experimental microorganisms to detect the inhibitory activity of plant extracts, as shown in table (1).

Table 1. Bacterial strains used for antibacterial activity tests

Bacterial strain	Source
Escherichia coli	College of Science – University of Baghdad
Staphylococcus aureus	College of Science – University of Baghdad
Salmonella typhimrum	College of Science – University of Baghdad
Klebseilla sp.	College of Science – University of Baghdad
Bacillus sp.	College of Agriculture – University of Tikrit

The method mentioned by [13] was followed to activate test microorganism strains and examine the susceptibility of them to the extract. The diffusion method in wells [14] was used to study the effect of both typical dill extracts. Holes with a diameter of 6 mm were made on the surface of the culture medium using a cork drill. Two concentrations of extracts (20% and 80%) were prepared. Dill plant extracts were added individually at a volume of 0.1 cm^3. Each plate was prepared with a hole containing sterile distilled water as control. The plates were incubated at 37°C for 18-24 h. The diameters of the inhibition zones around holes were measured. Two replicates were performed for each treatment.

Prepatin of biscuits: The biscuits were manufactured according to [15] with some minor modifications to suit experimental conditions. The following ingredients were used in the manufacturing process: 340 g of flour (with partial substitution of 5% and 10% with dill powder), 100 g of fat, 100 g of sugar, 20 g of skimmed powdered milk, 40 g of fresh eggs, 3 grams of sodium bicarbonate, 2 g of salt, and 1.2 grams of vanilla, In addition to the amount of water needed, depending on the absorbency of the flour. Fat and sugar were thoroughly mixed using an electric mixer, then eggs and vanilla were added to the mixture with continuous mixing until well combined. The dry ingredients (Flour, powdered milk, salt, and sodium bicarbonate) were gradually added to the mixture with continuous kneading for one minute to form a firm, soft dough. The dough was then cut into discs using 6 cm diameter round cookie cutters. They were then baked in an electric oven at 170°C for 10 minutes. After baking, the samples were left to cool at room temperature (25 ± 2

°C), then stored in tightly sealed glass containers until chemical, sensory and microbiological tests were performed on them.

Physical analyses of biscuits: The diameter (width)of the biscuit (cm), the thickness of the biscuit (cm) were measured according to [16].

Sensory analysis of biscuits: Sensory evaluation of biscuit samples was conductedby a panel of trained specialists [17-18].

Statistical Analysis: Data were analyzed using the General Linear Model (GLM) produced in SAS statistical software (2001) [19] according to a completely randomized block design (CRD). Duncan's Multiple Range Test was used to compare mean values, with significance set atp ≤ 0.05.

RESULTS AND DISCUSSION

Chemical composition of dill: The chemical composition of dil is presented in Figure 1. Protein and fat contents were 3.26% and 2.10%, respectively, which are higher than values reported by [20]. Moisture content reached 81.34%, comparable to 80.39% reported by [21]. Carbohydrate content was 9.02%, while crude fiber content was 3.12%, slightly higher than the 2.48% reported by [20]. Ash content amounted to 0.96%.

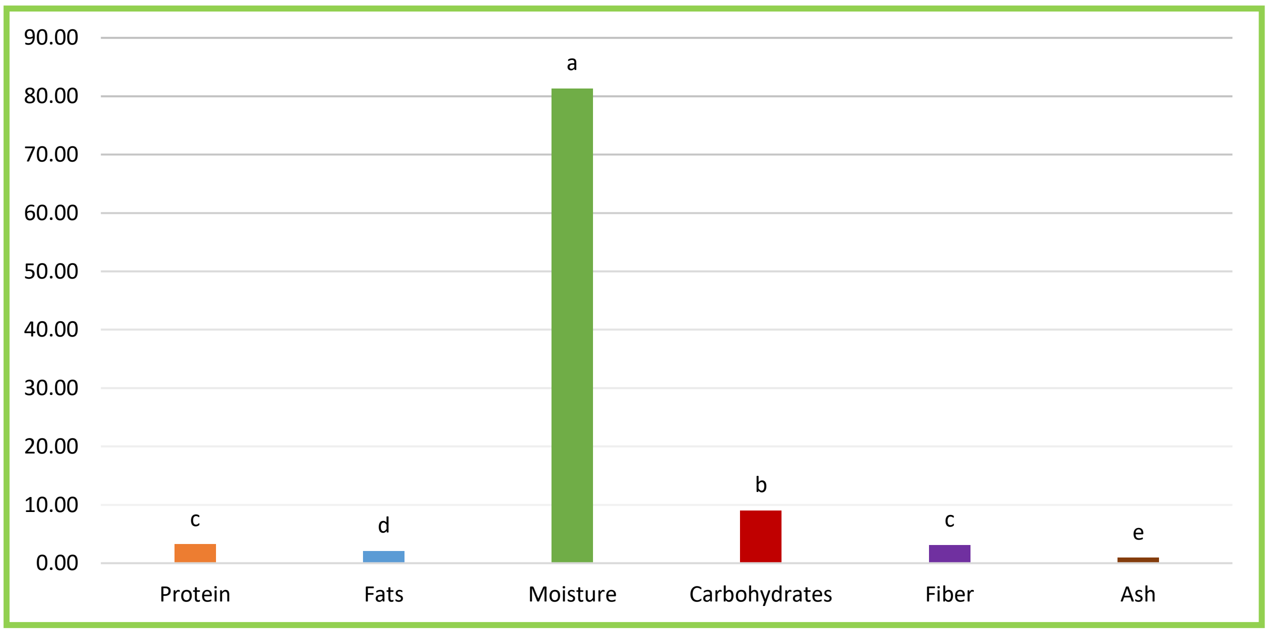


Figure 1. Chemical composition of the dill plant

The active compounds (phenols) of the dill plant: Analysis of the active compounds (total phenols) in dill revealed a wide range of distinctive phenolic compounds. As shown in Table 2, chromatographic analysis produced well-resolved peaks from 2.5 to 25 minutes, reflecting the chemical diversity of the plant. This profile highlights the richness of bioactive compounds, including flavonoids and tannins, which are known for their antioxidant activity. The presence of twelve major phenolic compounds is indicated by the chromatogram in dill plant, where it can be inferred that chlorogenic acid has the longest retention time (17.164 min), indicating less polarity, whereas procyanidin appeared earliest, consistent with its higher polarity. Among the identified compounds, quercetin showed the highest peak area (3173.63 V/S), indicating its abundance in dill (Table 2).

Table 2. Identified active compounds (phenols) of dill plant

compound name	Retention time (minutes)	Area (volts/second)	Quantity of compound (mg)	The main function of the compound
Procyanidins	2.356	1378.14	53.394	A powerful antioxidant, it inhibits free radicals and promotes lipid stability.
Callic acid	3.888	173.68	8.140	Effective antioxidant and antibacterial agent
Rutin	4.280	332.39	29.988	It improves capillary permeability and acts as an anti-inflammatory.
Kaempferol	5.408	1572.63	166.291	It has antioxidant and anticancer activity.
Cinnamic	8.256	3276.90	237.679	It inhibits microbial oxidation and improves aromatic taste.
Catechin	8.352	1523.37	284.73	One of the important flavonoids with antioxidant activity
4-hydroxy benzoic	9.824	2105.30	587.45	Natural antioxidant and bacterial growth inhibitor
Quercetin	10.072	3173.63	500.079	The most powerful antioxidant, it protects cell membranes from oxidation.
Cinnamaldehyde	10.388	5082.78	406.237	A powerful antimicrobial agent that disrupts the cell wall of microorganisms.
Eugenol	13.720	1407.13	174.297	An aromatic compound with antibacterial and antifungal properties.
Lignan	16.976	350.213	54.023	A plant-based phenolic compound with anti-cancer properties
Chlorogenic	17.164	311.048	58.636	It reduces oxidation and contributes to the stability of the food product.

The findings revealed that dill possesses an exclusive profile of active phenolics such as quercetin, catechin and kaempferol. In addition, its antimicrobial activity is probably associated with the existence of substances such as eugenol and cinnamaldehyde, which may justify dill use as a natural food preservative. On the other hand, 4-Hydroxybenzoic acid and cinnamic acid showed the lowest peak areas, indicating their low concentrations in dill. Collectively, these results indicate that dill involves numerous phenolic and aromatic compounds with potent biological activities, and that dill is a potential natural source of antioxidants.

These results are consistentwith other authors [22-23], who found that plant phenol-rich extracts are also very effective in scavenging oxidation and microbiological growth. Moreover, results from [24] indicated that higher phenolic content has a strong capacity to protect the food against oxidation and prolong shelf life which may also validate that dill can serve as a natural ingredient and an environmentally friendly substitute for synthetic chemical additives in functional food and food preservation applications. Hence, incorporation of dill powder or its extract can be suggested in the bakery products like biscuits and bread to increase their nutritive value as well as improve their sensory and physical characteristics [22,23]. Natural antioxidants extracted from plants, especially phenolic ones such as quercetin and catechin, have attracted attention. There has been significant interest recently due to the high demand for their use as an additive or dietary supplement [25].

Amino acid analysis: The amino acids of the dill plant were analyzed at different times (0, 5, 10, and 15 minutes) as shown in Figure (2). The results showed that the ratios of essential and non-essential amino acids varied across different time periods, with essential amino acids having the highest ratio. Results are reported at the 15-minute mark. It demonstrates that the content of glutamic acid is higher than other amino acids, also indicating that they play a crucial role in the protein structure of dill plant, which is 42.2% at retention time of 7.872 min. This discovery adds the nutritional value of utilizing proteins extracted from this plant. Glutamic acid is followed by Histidine (13.5%) and Phenylalanine (12.7%). It means that dill is not only rich in non-essential amino acids but also involved with essential amino acids necessary for humans. Meanwhile, moderate inclusive levels of other amino acids in the form of arginine, cysteine, tyrosine, isoleucine, and leucine were present (between 5.1% and 8.8%). The lowest percentage of amino acids was for cysteine, which reached 5.1 at a time of 11,200 minutes. The variation in amino acid ratios is attributed to differences in their degree of hydrolysis or partial stability during the periods of analysis. The results confirm that dill has a balanced composition of essential and non-essential amino acids. This makes it an important source of high-quality plant protein, and nutritionally, it has a good content of essential amino acids such as histidine, isoleucine, leucine, and phenylalanine This enhances the potential use of dill as part of a plant protein that can be utilized in human or animal nutrition [26]. Especially in the context of enhancing nutritional value and reducing reliance on animal protein sources, dill has been given great importance as an important medicinal plant [27-29].

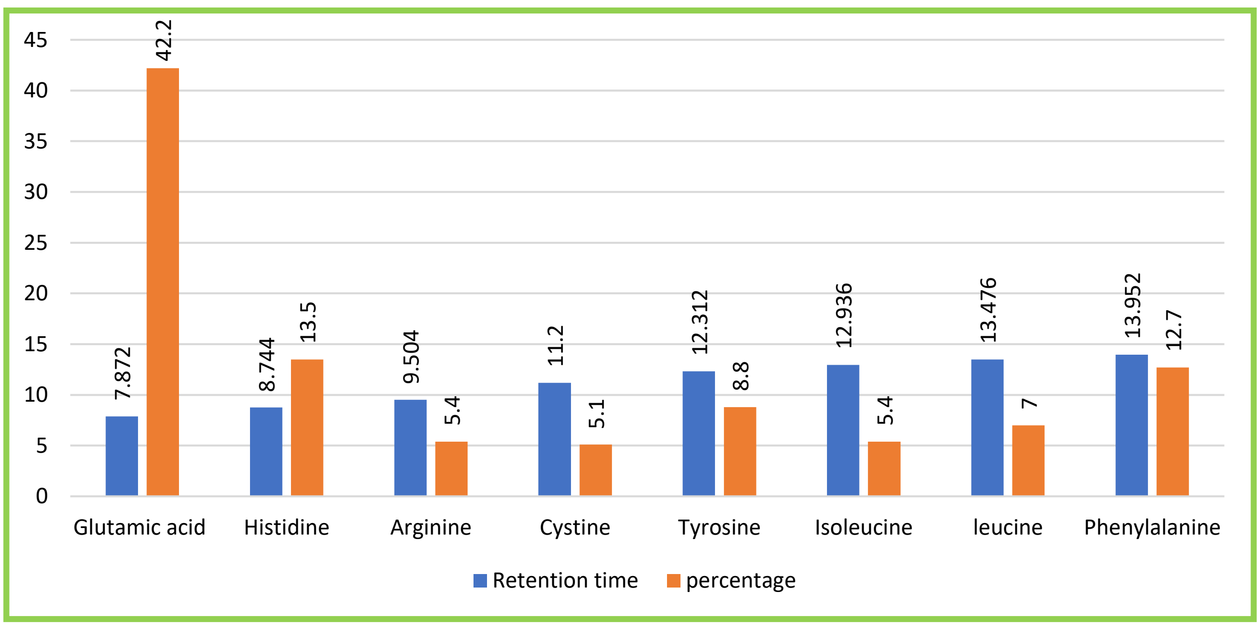


Figure 2. Amino acids of the dill plant

Microbiological tests: Five different types of experimental bacteria were used in this study, including three Gram-negative; *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella sp*. and two Gram-positive: *Staphylococcus aureus* and *Bacillus sp*. These microorganisms were chosen because it is a common cause of some diseases that affect humans and animals, and they are also considered a pollutant that causes spoilage of some foods and their resistance to antibiotics. The results in table (3) show that the aqueous dill extract has no antimicrobial effect. Some authors [30] mentioned that Dill extract has activity against organisms.

Table 3. Antimicrobial properties of aqueous extract of dill plant.

Concentration of aqueous extrat	Diameter of the inhibition zone				
	Staphylococcus aureua	*Escherichia coli*	*Bacillus sp.*	*Klebsiella sp.*	*Salomonella typhimurium*
20 %	-	-	-	-	-
80 %	-	-	-	-	-

-, no inhibition zone can be measured

While figure (3) and image (3) show the bacterial inhibition with the alcoholic extract of dill plants. All types of bacteria were sensitive, and the lowest sensitivity was noted for *E. coli* with inhibition zones reached 6.5 and 12 mm at concentrations of 20% and 80%, respectively. The results are similar to those found by [31], where they obtained a zone of inhibition of 13 mm. For Staphylococcus, the zone of inhibition was 12 mm. At a concentration of 20%, and Salmonella at a concentration of 80% (15 ml). These results were like what was found by [31]. The inhibition or elimination of microorganisms is attributed to several biological mechanisms, most notably the disruption of cell wall, formation or induction of disturbances in physical structure, or the chemical breakdown of proteins can lead to disruption of essential cellular processes. The inhibitory effect can also result from damage to the permeability of cytoplasmic membranes. This disrupts the balance of ions and substances within the cell. Furthermore, it may impair vital enzyme activities or inhibit protein and nucleic acid synthesis processes, leading to cessation of cell growth and death of microorganisms. [32-34].

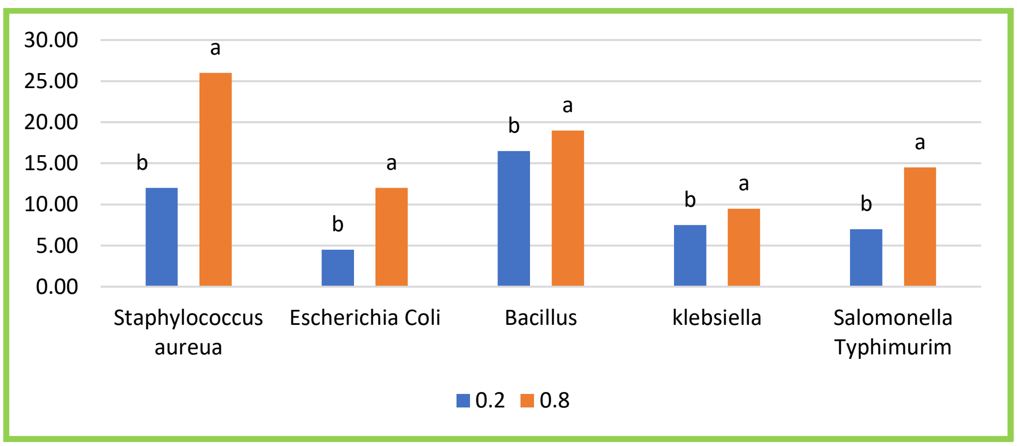


Figure 3. Antimicrobial properties of the alcoholic extract of dill plant

Image 3. Bacterial inhibition with the ethanolic extract of dill plant

Physical properties of biscuits: The results in Figure (4) show mixtures 1 and 2 comparing with standard sample. Mixture 2 recorded better results than the standard mixture and the diameter increased with increased addition in large quantities, because the characteristic of spreading is due to the presence of fibers in dill.

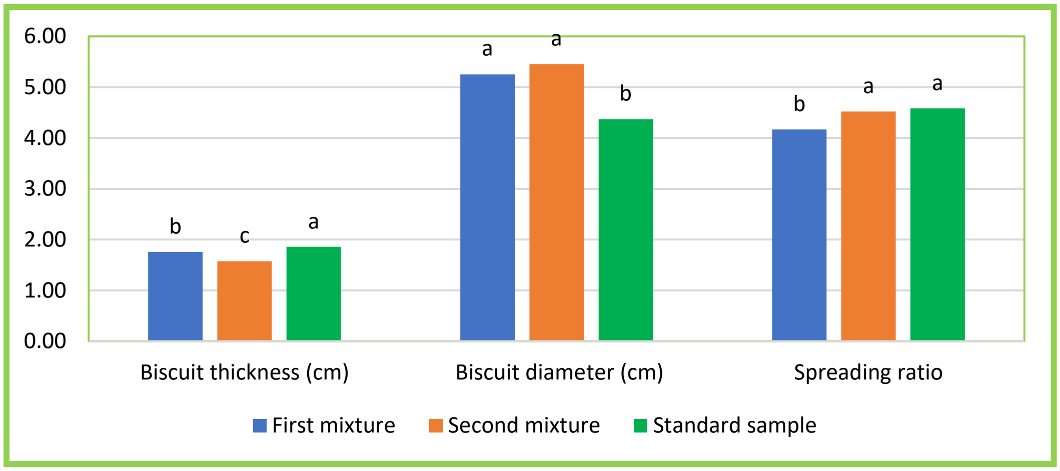


Figure 4. Physical properties of biscuits

Sensory assessment: The results of sensory evaluation of biscuits are shown in Figure (5). The sensory qualities studied (general appearance, top surface quality, freshness, crunchiness, flavor, aroma, taste, color and overall acceptability). For biscuits made from three treatments: standard mix and substitute flour with 5% and 10% dill powder. The second mix (10% dill powder) was better than the first, with a slight statistically significant difference. It can be adopted by people with digestive disorders. This product can be considered as good because it represents a good source of many essential vitamins and minerals. The biscuits were evaluated by a number of evaluators (10) based on qualitative characteristics (color, smell, texture, taste). As shown in the Image (4), biscuits made from wheat flour with added 5% and 10% dill were almost similar.

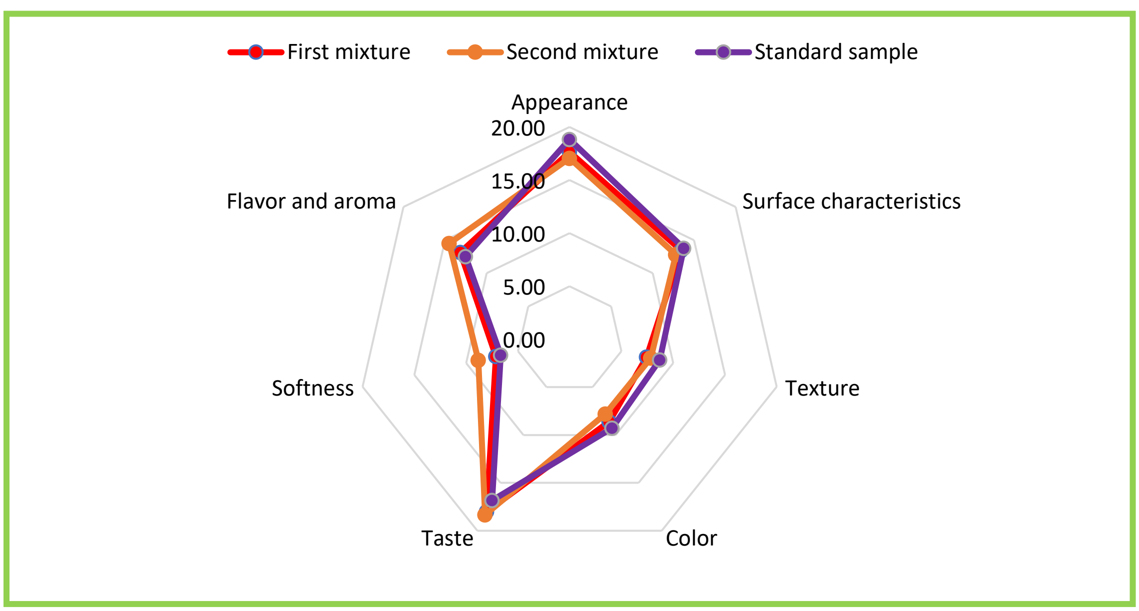


Figure 5. Sensory evaluation of the produced biscuits. (Mixture 1) Biscuits made from wheat flour and 5% dill. (Mixture 2) Biscuits made from wheat flour and 10% dill

Image 4. Biscuits made from wheat flour only and from wheat flour substituted with 5% and 10% dill powder

This work presents a novel contribution by demonstrating the successful incorporation of dill powder into a bakery product as a multifunctional ingredient. Previous studies have primarily focused on dill extracts for their antimicrobial or antioxidant properties, while others have investigated fiber fortification of biscuits using different plant materials. However, the combined effects of dill powder as both a nutritional enhancer and a natural preservative in biscuits, representing the primary objective of this study, have not been previously reported.

The present work shows that dill powder not only increases fiber, phenolic content, and antioxidant activity, but also imparts measurable inhibitory effects against key foodborne bacteria such as E. coli, Salmonella typhimurium, and Staphylococcus aureus. This dual functionality distinguishes the developed biscuits from conventional fortified products that rely on synthetic preservatives.

Moreover, using whole dill powder rather than purified extracts enhances sustainability, cost-effectiveness, as well as practical applicability for the food industry. In regions where dill is locally abundant. Therefore, this study establishes a new approach for producing functional, plant-based bakery products with extended shelf-life and improved nutritional value using a single natural ingredient

Conclusions: Dill exhibits valuable nutritional properties, including appreciable levels of proteins, fats, and other essential components, underscoring its significance as a natural source of phytonutrients. The alcoholic extract of dill demonstrated stronger antimicrobial activity than the aqueous extract, particularly against *E. coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. These findings highlight the multifaceted potential of dill as a rich source of phenolic compounds and bioactive amino acids. Consequently, dill represents a promising ingredient for the functional food industry, serving as a natural antioxidant and antimicrobial agent that can safely enhance the nutritional value, sensory attributes, and overall quality of food products.

Authors Contribution*:* Noor Jumhaa Fadhil: Formal analysis and Methodology; Validation and Writing original draft. Baraa A almairza Project administration; Abeer Majeed Funding acquisition. Sara Thamer Hadi: Data duration; Formal analysis and Methodology.

Competing Interests: The authors declared no conflict of interest.

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